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Survival probability of epigenetically defined IDH-wild-type glioblastoma without necrosis or vascular proliferation

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We report that (epi)genetically classified histological glioblastoma (GBhisto) and molecular glioblastoma (GBmol) do not significantly differ in their overall survival when cohorts are stratified for combined radiotherapy and chemotherapy treatment. Interestingly, in our cohort, there was no survival benefit of MGMT promoter methylation in GBmol patients. When examining clinicopathological parameters more closely, GBmol often show no contrast enhancement, are less frequently resected, and exhibit a different composition of DNA methylation subclasses compared to GBhisto.

The update of the *WHO Classification of Tumours of the Central Nervous System* no longer mandates necrosis or vascular proliferations for the diagnosis of glioblastoma (GB) if specific molecular features such as gain of chromosome 7 with combined loss of chromosome 10 (+7/–10), *EGFR* amplification, or *TERT* promoter mutation are present.[1] This has been implemented by cIMPACT-NOW as former studies suggested similar clinical courses of IDH-wild-type GB (WHO grade 4) and IDH-wild-type diffuse astrocytic gliomas WHO grade 2/3.[2] First analyses of tumors that now can be reclassified as GB (in the absence of classical histological features, molecular glioblastoma [GBmol]) revealed potentially important clinical differences including a lack of benefit from temozolomide regardless of *MGMT* promoter methylation status.[3] Additionally, a significant overall survival benefit in favor of GBmol with lower-grade histology in comparison to grade 4 histological GB counterparts (GBhisto) was reported.[4] Another study revealed a higher percentage of epilepsy and a lower percentage of resected tumors among GBmol, while no differences in overall survival were detected.[5]

We here assembled a multicenter retrospective cohort of diffuse gliomas without necrosis or vascular proliferation that, in contrast to the aforementioned studies, had been classified by DNA methylation analysis and copy-number profiling with either a calibrated score (classifier version v11b4) of ≥0.84 for the diagnosis of GB, IDH wild-type, or with a score of ≥0.7 and presence of +7/–10 or *EGFR* amplification (GBmol cohort, $n = 50$).[6] A cohort of cases with histological hallmarks of GB was used for comparison with a calibrated score (classifier version v11b4) of ≥0.84 for the diagnosis of GB, IDH wild-type (GBhisto cohort, $n = 209$). In addition to the DNA methylation class and copy-number profiles, the MGMT promoter methylation status was also extracted from the methylation data according to the logistic regression model (MGMT-STP27) by Bady et al.[7] The study protocol was approved by the institutional ethical board (#SNO-9-2021).

Based on the methylation classifier v12.7, we removed 6 GBmol and 5 GBhisto cases that matched pediatric-type high-grade gliomas (Supplementary Figure 1A). After the exclusion of these cases, the GBmol and GBhisto cohorts finally comprised 44 and 204 cases, respectively (Supplementary Figure 1B). Of these 248 patients, 141 patients received combined radiotherapy and chemotherapy (RT/CT) (Figure 1A).

GBmol were less likely to match within the RTK1 methylation subclass and were more likely to match within the mesenchymal subclass (Supplementary Figure 1C). The 2 cohorts were overall balanced in respect of age, Karnofsky performance status (KPS), sex, and *MGMT* promoter methylation status (Supplementary Table 1). Further, GBmol were more likely to be diagnosed by biopsy (Supplementary Figure 1D). A lack of contrast enhancement was found in approximately one-third of GBmol and in less than 2% of GBhisto tumors indicating a clear difference between these 2 groups (Figure 1B). Contrast enhancement was associated with worse outcomes in the entire cohort (Figure 1C).

Patients who received a biopsy in the GBmol cohort had a significantly better preoperative KPS as biopsy patients of the GBhisto cohort (Supplementary Figure 1E). Significant differences in the overall survival of GB patients were observed depending on RT/CT treatment (Supplementary Figure 1F, G). Comparing GBmol and GBhisto cases receiving RT/CT revealed no significant differences in survival, albeit especially in the cohort of biopsy-only cases, a favorable trend was detectable (Figure 1E, F). Without therapy stratification, we observed

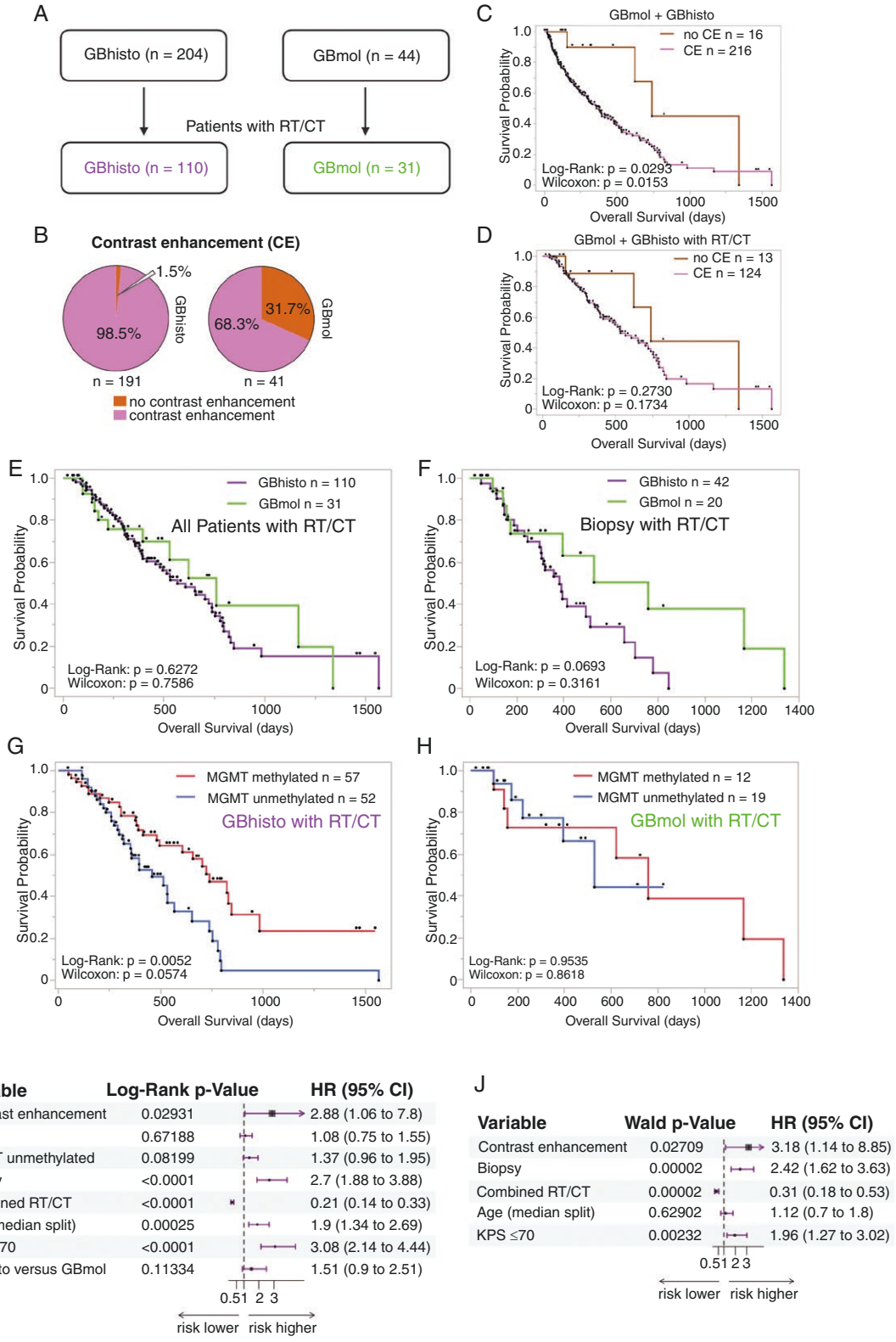

Figure 1. (A) Stratification of both cohorts by RT/CT (missing data for 42 patients). (B) Distribution of the variable "contrast enhancement" in both cohorts. Overall survival with regard to "contrast enhancement" (CE) in the entire cohort (C) and in patients that received RT/CT (D). Kaplan–Meier survival curves of the cohorts included only patients who received RT/CT (E) and patients who received RT/CT and tumor biopsy (F). Kaplan–Meier survival analysis of GBhisto (G) and GBmol (H) subject to *MGMT* promoter methylation status (all patients treated with RT/CT). Univariate (I) and multivariate (J) Cox proportional Hazards Model of the entire cohorts GBmol + GBhisto. Variables that reached statistical significance ($P < .05$) in univariate log-rank test were subjected to multivariate analysis.

a potential survival advantage for the GBmol cohort (Supplementary Figure 1H, I).

A recent study suggested a lack of benefit from temozolomide treatment regardless of *MGMT* promoter methylation status in GBmol.[3] Similarly, in our cohort, improved survival of GB patients with *MGMT* promoter methylated tumors was exclusively observed in the GBhisto cohort (Figure 1G, H; Supplementary Figure 1J, K). Ultimately, when both cohorts were combined, the variables contrast enhancement, extent of resection, RT/CT, age, and KPS ≤ 70 emerged as significant risk factors in univariate Cox proportional hazards analysis. When including these variables in a multivariate Cox proportional hazards model, all variables except age showed a statistically significant impact on overall survival (Figure 1I, J).

The logistic regression model for the assessment of MGMT promoter methylation status includes limited CpG sites for evaluation. This can be considered a limitation of our study. However, this does not explain the different results in the GBhisto and GBmol cohorts.

As a novel finding, we report that RTK1 in contrast to RTK2 and mesenchymal subclass is under-represented among GBmol. With regard to *MGMT* promoter methylation status and GB DNA methylation subclass, differential clinical outcomes have already been described.[8]These data demonstrate an absence of survival benefit from *MGMT* promoter methylation in RTK1 and mesenchymal subclass but prolonged survival in *MGMT*-methylated RTK2 GB.[8] Another study revealed that the extent of resection did not influence survival in mesenchymal in contrast to RTK1 and RTK2 subclass GB, suggesting that the methylation subclass could have important consequences for treatment response and benefit from the extent of resection.[9]

Our work, as well as the studies mentioned above, demonstrates the importance of including DNA methylation analyses in the diagnostic workup of brain tumor cohorts.

Our analysis with a focus on histopathology and application of DNA methylation classifiers adds to the increasing knowledge that GBmol might be associated with a clinically distinct disease course with similar overall survival as GBhisto but differences in the predictive value and potentially prognostic value of MGMT gene promoter methylation. Our study is limited by small sample size and the retrospective nature as well as the lack of specific analysis of TERT promoter mutations. Future trials, especially prospective studies, will be necessary to further define prognostic and predictive characteristics of GB diagnosed in the absence of classic histology.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Advances* (https://academic.oup.com/noa).

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Conflict of interest statement

All authors declare no conflicts of interest with the content of the present study.

Authorship statement

Concept of the study and supervision: P.N.H., J.P.S., D.C., M.W.R. Provided material, methods, or data: P.N.H., K.J.W., F.R., R.D., U.S., M.H., T.H., H.D., T.A., A.v.D., M.H., I.D., K.U., J.P.S., D.C., M.W.R. Performed data analysis: P.N.H., K.J.W., F.R., R.D., U.S., M.H., T.H., I.D., K.U., M.W.R. Drafting of the manuscript: P.N.H., J.P.S., D.C., M.W.R. Editing, reviewing and approval of the manuscript: P.N.H., K.J.W., F.R., R.D., U.S., M.H., T.H., H.D., T.A., A.v.D., M.H., I.D., K.U., J.P.S., D.C., M.W.R.

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